boiled with dilute acid in order to hydrolise the glucoside, considerable oxidation, accompanied by brown coloration, will take place.

Palladin* maintains that the formation of *post-mortem* pigments from aromatic chromogens is proof of the significance of the latter in respiration. Some of the plants from which he obtained the greatest quantity of pigment by treatment of the extracts with peroxidase and hydroxyl are of the pyrocatechin-containing type, and the presence of this phenol, when it occurs, would doubtless accelerate the oxidation of the extracts. But the reactions obtained after death may be no real guide to knowledge of the true metabolic reactions of the living tissues.

The formation of brown pigment on autolysis and injury in pyrocatechin-containing plants is no doubt largely due to the oxidation of the phenol itself, but, in addition, coloration may be caused by the oxidation of other aromatic compounds, *i.e.* tannins, flavones, etc., when once the system peroxide-peroxidase has been established.

The Action of Radium Radiations upon Some of the Main Constituents of Normal Blood.

By Helen Chambers, M.D., and S. Russ, D.Sc., Beit Memorial Research Fellow.

(Communicated by Dr. J. R. Bradford, Sec. R.S. Received May 1,—Read June 1, 1911.)

The following experiments were undertaken with a view to determining the effect *in vitro* of the different radiations from radioactive substances upon some of the main constituents of normal blood. The observations have so far been extended to the hæmolytic action of the α-rays on red corpuscles, to the effect of these rays on leucocytes, and to their action on opsonin and complement. Numerous experiments have also been made with the β- and γ-rays, but, generally speaking, the results have been of a negative character.

The Hæmolytic Action of the Emanation.

When radium emanation is mixed with citrated human blood, hæmolysis results. The liberation of hæmoglobin is a gradual process, as is evidenced

* Palladin, "Über das Wesen der Pflanzenatmung," 'Biochem. Zeitsch.,' 1909.

by the following experiment, which is typical of several:—A glass bulb of volume 30 c.c. contained 2 c.c. of citrated blood and emanation equal in quantity to the equilibrium value of 26·5 mgrm. $RaBr_2$. The blood was examined at different times and a count made, by means of a Thoma Zeiss apparatus, of the percentage of completely hæmolysed corpuscles. The results are indicated in Table I.

Table I.

Time of exposure.	Completely hæmolysed corpuscles. Percentages.
hours.	
2	0
6	0
19	7·2
$42\frac{3}{4}$	84

When hæmolysis was complete, the corpuscles were found to be colourless and slightly shrunk, but retaining their corpuscular form. The spectrum of met-hæmoglobin was observed at the end of the observations. The conversion from oxy-hæmoglobin was not complete, however, as some of the bands of this substance were also visible.*

The hæmolysis was found to be due to a direct action of the α-particles on the red corpuscles by the following experiments:—

(1) Emanation mixed with washed red corpuscles gave marked hæmolysis in 24 hours.

(2) Emanation mixed with serum for 24 hours and the latter added to washed red corpuscles gave no hæmolysis.

(3) Emanation enclosed in a glass tube just thick enough to exclude the α-rays, while allowing free exit of the β- and γ-radiations, produced no hæmolysis in blood contained in a tube which surrounded that in which the emanation was held.

The concentration of the emanation in these three experiments was nearly the same as in that initially described. The direct proof of the hæmolysis being due to the α-particles has been shown by means of the apparatus of fig. 1.

A finely powdered specimen of radium bromide was spread over a circular area of 2 sq. cm., this being the bottom of a cavity 1 mm. deep in a

* Hæmolysis and the formation of met-hæmoglobin have been observed by Henri and Mayer ('Comptes Rendus,' 1904, p. 521) experimenting with frog's blood and that of the dog, by exposing it to 100 mgrm. of radium. The type of radiation producing the results is, however, not stated.

brass capsule (fig. 1). The grains were held in position by very thin varnish. The cavity was covered with an air-tight sheet of mica (A), sufficiently thin to allow escape of the α-particles.*

On another thin sheet of mica (B) a drop of citrated blood (C) was spread over a known area. The drop was covered over with a shallow watch-

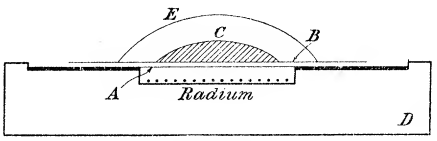

FIG. 1.

glass (E), vaselined round its edge to prevent evaporation. The mica B was then placed over A, and radiation proceeded for any desired interval.

It was clear that, since the liberation of hæmoglobin is a gradual process, if an accurate relation between the time of radiation and the number of hæmolysed corpuscles were to be found, sufficient time must elapse after radiation and before the count was made, to allow of the release of the hæmoglobin from the affected corpuscles. Twenty-four hours were found to be sufficient for this purpose.

The same volume of citrated blood (12·5 c. mm.) was taken each time and spread over an area of 1·5 sq. cm. on a sheet of mica. This was then exposed to the α-radiation from the radium capsule. A control was provided in each case.

It may be seen from Table II and the curve in fig. 2 that the number of totally hæmolysed corpuscles for a given intensity of radiation bears a simple relation to the time of exposure. A separate experiment showed that the products of hæmolysis had no hæmolytic action on other red corpuscles.

Table II.

Time of exposure.	Percentage of unhæmolysed corpuscles.
h. m.	
0 15	98
1 0	93·5
1 40	84·5
4 15	58·8
6 35	45·6
10 45	25
24 0	10

* Two such brass capsules were made, one containing 2·4 mgrm., the other 3·27 mgrm. $RaBr_2$. We are indebted to Mr. F. H. Glew for their preparation.

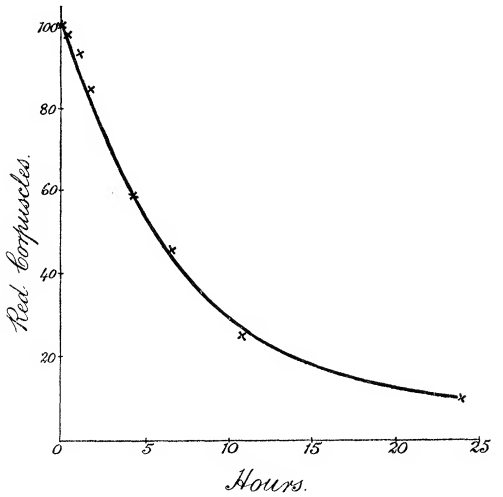

FIG. 2.

An estimate of the number of α-particles required to hæmolyse a red corpuscle is possible owing to the precision with which the essential quantities in the calculation are known, viz., the number of corpuscles per cubic millimetre of blood, and the number of α-particles emitted per second from a measured quantity of emanation.*

From experiments in which the emanation was mixed with blood, a calculation results in the number 2000 being required for the complete hæmolysis of a red corpuscle. From those in which the α-particles had to penetrate two sheets of mica, a maximum estimate of the number in question is 8000. In view of the different experimental conditions, the difference between the two numbers is not significant.

The Action of the α-Rays on Leucocytes.

It has been found that the α-particles are capable of not only destroying leucocytes, but also, by virtue of their action on the serum, rendering a radiated region free of them.

A simplification of Ponder's† method of obtaining leucocytes has been used for these experiments. A drop of blood is put on a mica plate, covered, but not touched, by a watch-glass to prevent evaporation, and incubated at 37° C. for about 20 minutes. On removal of the clot large numbers of leucocytes are found on the surface of the mica, to which they

* Rutherford and Geiger, 'Roy. Soc. Proc.,' A, 1908, vol. 81, p. 173.

† Ponder, 'Camb. Phil. Soc. Proc.,' 1909, vol. 15, Part I.

adhere firmly; they may be repeatedly washed and then stained without being freed.

If the blood be not incubated, clotting is delayed, and the motion of the leucocytes to the surface considerably prolonged; this is of importance in the following experiments:—

A drop of blood was placed on a clean piece of mica, sufficiently thin to allow easy penetration of the α-particles. It was covered with a watch-glass and placed on the radium capsule, the radiation from which was screened in such a manner that it was entirely confined to a square window of about 1·5 sq. mm. On placing the mica sheet over the capsule, therefore, the drop of blood was not radiated by the α-rays except the area which was directly above the small window.

After an exposure of about 20 hours *at room temperature* the clot was removed, the mica surface washed with saline, and stained. The resulting picture was as indicated by fig. 3.

It is seen that in a region corresponding to the radiated area there is an almost complete absence of leucocytes; the area free of leucocytes is, however, slightly greater than that of the window, the ratio determined by a magnified projection on squared paper being 1·34 : 1. This increase is probably due to cross firing of the rays. A different result was obtained under the following circumstances:—

A drop of blood was shed on to a thin mica sheet, covered with a watch-glass, and *incubated for* $1\frac{1}{2}$ *hours.* This ensures a plentiful supply of leucocytes on the mica surface.

The system was removed from the incubator and placed over the radium capsule, which was again provided with a small square window, of area 1 sq. mm., through which came the α-rays. After an exposure of about 20 hours at room temperature, followed by the usual staining process, the picture presented was as shown in fig. 4.

Inspection showed that the area free of leucocytes was much larger than that of the window. A measurement similar to that already described gave the ratio 2·8 : 1. In this experiment numerous degenerate leucocytes were observed in the radiated and surrounding zones.

If therefore the α-radiation proceeds during the slow migration of the leucocytes out of the clot to the mica at room temperature, the area free of leucocytes practically corresponds to the aperture through which the rays come. If, however, the leucocytes are first allowed to make their way to the mica surface, part of which is then radiated, the area free of leucocytes is found to be much larger than that of the radiated area.

The different results in these two experiments indicate that in the former

the leucocytes do not reach a radiated region, but tend to drift into a protected zone. The leucocytes do not, however, move as a direct result of the α-radiation, for if incubation occur simultaneously with radiation, leucocytes are found on the radiated surface, although to a modified extent.

During the slow motion of leucocytes to the mica at room temperature, changes are taking place in the radiated serum, forming a layer over the mica. As will be seen later, there is a lowering of the opsonin and complement content of the serum over the radiated region.

The leucocytes seem to move from a radiated to a non-radiated region, *i.e.*, from a serum in which changes have been induced by the radiation, to a serum which is unaltered. This motion can be explained by changes in

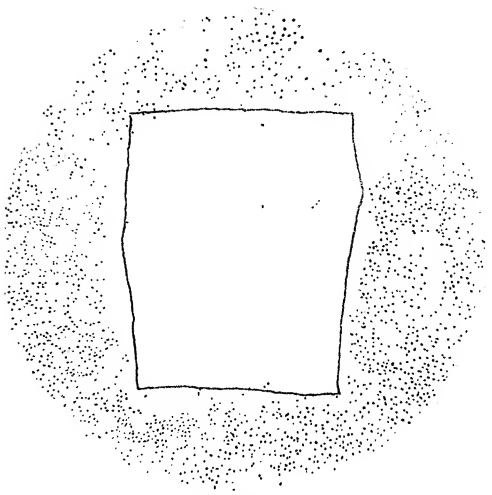

FIG. 3.

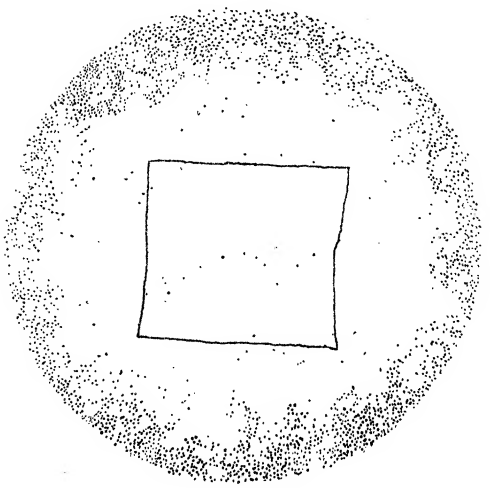

FIG. 4.

surface tension corresponding to some alteration in the constitution of the fluids in which the leucocytes are moving.

The surface tension of α-radiated serum, determined by the capillary tube method, showed a reduction when compared with that of a control normal serum. The change is indicated by the figures in Table III.

Table III.—Surface Tension in dynes per cm. at about 10° C.

Normal serum.	Radiated serum.	Time of radiation.
		hours.
66·2	63·2	24
66·2	61·2	44
67·5	59·1	54

Further evidence of the absence of leucocytes upon a radiated surface and of their drift into a protected region is given by fig. 5, which was obtained by screening the α-radiation from one-half of the capsule. The motion of the leucocytes occurred at room temperature.

To account for the enlargement of the area free of leucocytes when incubation occurs previous to radiation, the suggestion is put forward that the leucocytes are destroyed after making their way to the mica surface and liberate some fluid products which have a destructive effect upon the leucocytes in the surrounding zones. These products gradually diffuse away

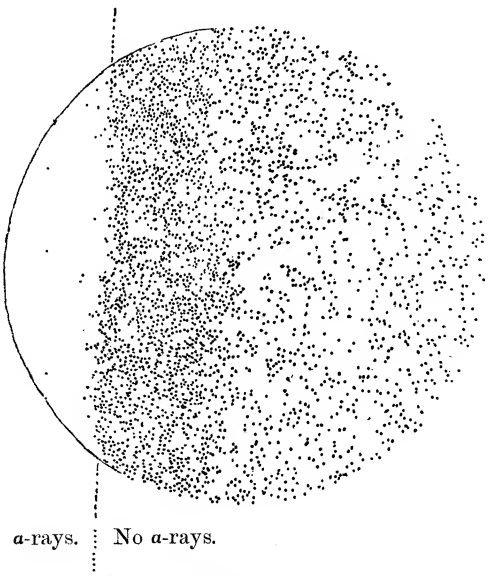

FIG. 5.

from the radiated area and effect the destruction of leucocytes quite outside the direct stream of the α-rays.

This observation was substantiated by a series of experiments in which apertures of different sizes were used. The diffusion effect, measured by the enlargement of the radiated region, was more pronounced the smaller the aperture.

The Action of the Radiations on Opsonin.

The experiment has been described in which citrated blood was exposed to the action of the emanation in a closed glass bulb and the degree of hæmolysis observed from time to time. Simultaneously with these observations the opsonic content of the blood was compared with that of the control.

The usual procedure in estimations of opsonin was adopted and an emulsion of *Staphylococcus aureus* was used in every case. The first estimation, which was made 19 hours after exposure of the blood to the emanation had begun, gave evidence of a reduction of the opsonic content. The leucocytes used had, however, been subject to the radiations, and were degenerated. In consequence of this action the radiated blood was centrifugalised and the opsonin content of the supernatant fluid was determined with freshly washed leucocytes.

After 44 hours' exposure the following result was obtained :—

Control fluid gave ..... 372 micro-organisms in 60 leucocytes.
Experimental fluid gave 34 „ 60 „

The effect, then, of long exposure is apparently a marked reduction in the opsonin.

According to some experiments by H. Reiter,* in which emanation was mixed with blood for short periods of time, an *increase* of the phagocytic power of blood cells was observed, for to quote from this author—"Soweit nach den Versuchen *in vitro* zu erteilen ist, scheint die Emanation die phagocytäre Tätigkeit der Blutzellen anzuregen, in einzellen Fällen bis zu 30%."

The experiment was varied, as, during the previous exposure, the serum was the recipient of products of radiated corpuscles, the action of which might possibly injuriously affect phagocytosis. Some citrated blood was centrifugalised and about 1 c.c. of the supernatant fluid was exposed to the emanation and examined on two occasions with the following results :—

Time of exposure.					
19 hours	Control fluid gave	495	micro-organisms in	160	leucocytes,
	Experimental fluid gave	84	„	160	„
42 „	Control fluid gave	388	„	100	„
	Experimental fluid gave	57	„	100	„

In order to test whether there were substances formed in the radiated fluid which might be inhibitory to phagocytosis, further observations were made with the two plasmas of the last experiment. The procedure is indicated as follows :—

Experimental plasma + Control plasma + Micro-organisms + Washed leucocytes.—Film I.
1 volume 1 volume 1 volume 2 volumes.
Saline solution + Control plasma + Micro-organisms + Washed leucocytes.—Film II.

* H. Reiter, 'Zentralblatt für Röntgenstrahlen, Radium, etc.,' 1910, p. 243.

A count of the two films which were prepared gave the following results :—

Film I gave 441 micro-organisms in 150 leucocytes.
Film II „ 541 „ 150 „

It is clear that if the experimental plasma had contained substances inhibitory to phagocytosis, their influence should still be manifest when added to the control plasma. The difference between the counts of the two films, amounting as it did to only 20 per cent., shows that the large reduction previously observed with the radiated serum is mainly to be attributed to a reduction in the opsonin normally present.

The effective agent is here again the α-particle. By excluding this type of radiation and exposing serum to the β- and γ-rays, no appreciable alteration in its opsonic content was obtained with quantities of the order 5 to 10 mgrm. $RaBr_2$, and for exposures lasting about forty hours.

A series of observations was made by taking a measured volume of serum (12·5 cu. mm.) spread over an area of 1·5 sq. cm. on a thin mica sheet and exposing it to the α-radiation from one of the capsules. A fresh sample of serum was used for each exposure, as, owing to the very limited penetration of the fluid by the α-rays, only just sufficient for an opsonic determination was radiated. After the serum had been radiated for any selected interval, its opsonic content was compared with that of the control.

The results obtained are given in Table IV and shown graphically in fig. 6 :—

Table IV.

Time of radiation.	Percentage of opsonin in radiated serum.	Number of micro-organisms in 100 leucocytes.	
		Control.	Experiment.
h. m.			
2 40	82	620	506
5 15	58	620	360
10 30	59	386	228
14 0	31 42 per cent.*	430	182
18 20	35 47 „ *	507	244
20 15	24	210	50
36 45	14	383	53
45 0	9	681	64

* A smaller quantity of Ra was used in these cases, the numbers on reduction give 31 and 35 per cent.

The general character of the curve is of the simple exponential type, but owing to the possibility that the method of estimating the amount of

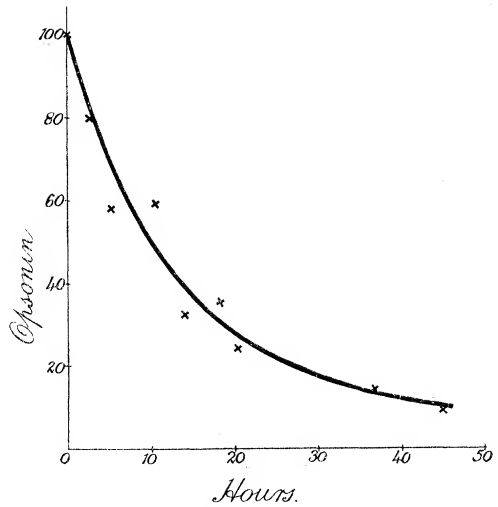

FIG. 6.

opsonin is not strictly quantitative, it cannot be asserted that the destruction of opsonin rigorously follows an exponential law.

The Action of the Radiations on Complement.

A similar series of observations was made with hæmolytic complement. For this purpose a thin film of serum was exposed to the α-radiation from one of the capsules in the manner already described. The complement in the radiated serum was then compared with that contained in the control.

In order to obtain a quantitative estimate of the complement, a method similar to that described by Dr. Emery* has been used.

Briefly, the method consists in procuring a 20-per-cent. suspension of fully sensitized red blood corpuscles, obtained by adding to 1 volume of washed corpuscles, 4 volumes of a strong immune serum. To 4 volumes of this suspension is added 1 volume of the serum, the complement in which is being tested. After being kept at 37° C. till hæmolysis is complete, the liquid is centrifugalised and the amount of free hæmoglobin in a constant volume (76 cu. mm.) of the supernatant fluid tested by means of a Sahli hæmometer.

This instrument was calibrated by adding to 4 volumes of sensitized red corpuscles 1 volume of serum of varying dilutions. The amount of free hæmoglobin as read from the scale of the instrument was found, within the limits of the experimental error, to be proportional to the strength

* Emery, 'Lancet,' 1911, p. 490.

of the serum added, and therefore to the amount of complement present, as may be seen from fig. 7. For hæmometer readings below 20 accuracy is not claimed.

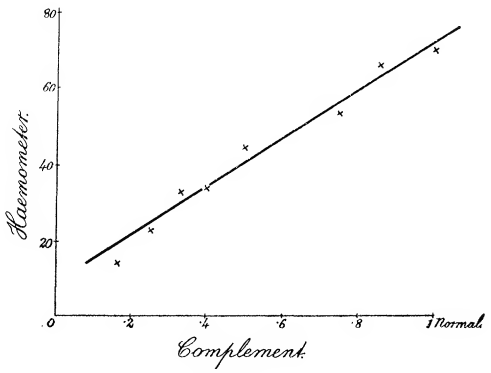

FIG. 7.

The volume of serum exposed to the radiation in the opsonin experiments was 12·5 cu. mm., but this being an inconveniently small quantity in the complement estimations, the volume was increased to 30·5 cu. mm. After an exposure of any desired interval the amount of complement in the radiated serum was compared with that in the control. The results may be seen from Table V and fig. 8.

Table V.

Time of exposure.	Percentage of complement remaining.
h. m.	
13 15	92
17 30	82·5
22 15	76·6
28 0	86
36 25	64
41 15	48
44 35	46·8
46 30	52·9
54 15	15 estimated
66 30	10 ,,

A comparison between this curve and that in fig. 6 shows a striking dissimilarity in the reductions of opsonin and of complement in serum when subject to α-radiation; but the two curves are not quantitatively comparable, owing to the difference in the volume of serum radiated in the two cases. For this reason three determinations of the reduction in opsonin

were made in which the same volume of serum was radiated as in the complement experiments. These three observations are indicated by the thin line curve, fig. 8. The type of the curve is identical with that

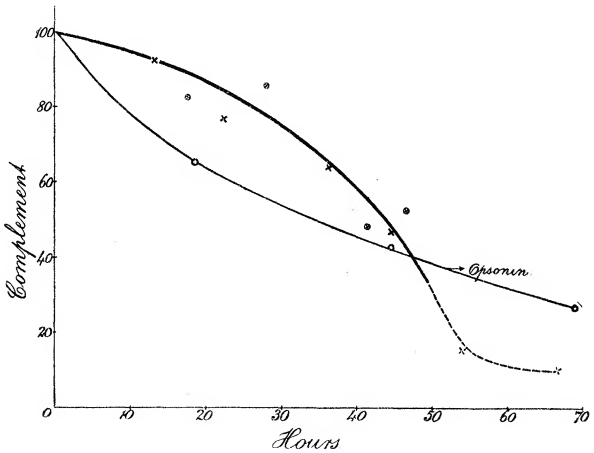

FIG. 8.—The crosses (×) on the complement curve indicate values obtained with the use of sensitized sheep's corpuscles, the circles (o) those obtained with sensitized human corpuscles.

previously obtained, the only difference being a diminished rate of reduction of opsonin, as was to be anticipated.

Inspection of the complement curve shows that the reduction is slow at first, the rate gradually increasing with time, as may be seen from the convexity of the curve to the time axis.

The experimental and control sera were, except for the radiation, kept under identical conditions at room temperature. The spontaneous disappearance of complement is therefore eliminated as a disturbing factor, by a comparison between the two sera. It should be pointed out, however, that fresh serum was used for every experiment, because it was found that the complement in serum which has been kept for some days at 0° C. was more affected by the radiation than fresh serum.

The general character of the two curves (fig. 8) indicates the separate identity of opsonin and complement.

The complement curve suggests either that this substance becomes more unstable under the action of the radiation, or that its amount is in some way dependent upon some other substance present in serum.

The small initial reduction in complement could, on the latter supposition, be explained if this substance under the action of the radiation were eventually reduced to complement; then, despite the simultaneous reduction

in the latter, a supply would be provided owing to the breaking down of the former.

Our present methods need elaboration before this question can be settled.

Summary of Conclusions.

1. Red blood corpuscles are hæmolysed by the action of α-rays, and oxy-hæmoglobin is converted into met-hæmoglobin.

2. Leucocytes undergo marked degenerative changes when subjected to α-rays. During the process of clotting, leucocytes appear to move away from an α-radiated region. This movement has been attributed to changes found to occur in the surface tension of blood serum when radiated.

3. The specific properties of opsonin and hæmolytic complement are lost when serum is exposed to α-rays. The progressive changes caused by these rays indicate the separate identity of opsonin and complement.

4. The β- and γ-rays have yielded negative results in analogous experiments.

On a New Method of Estimating the Aperture of Stomata.

By Francis Darwin, F.R.S., and D. F. M. Pertz.

(Received June 15,—Read June 29, 1911.)

CONTENTS.

§ 1. *Method.*

It is usually assumed that transpiration is regulated by two principal factors: (1) the relative humidity of the air, and (2) the degree of aperture of the stomata. Neither of these assumptions has been experimentally proved, though both of them are necessarily true, but it must be remembered that the factors referred to are not necessarily the only ones that govern the phenomena.

The experiments hitherto made on (1) the effect of relative humidity are